

An easy microwave-assisted synthesis of C8-alkynyl adenine pyranonucleosides as novel cytotoxic antitumor agents

Athina Dimopoulou¹, Stella Manta¹, Vanessa Parmenopoulou¹, Nikolaos Kollatos¹, Ourania Christidou¹, Virginia V. Triantakou², Dominique Schols³ and Dimitri Komiotis^{1*}

¹ Laboratory of Bioorganic Chemistry, Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece, ² Laboratory of Organic Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece, ³ Department of Microbiology and Immunology, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

OPEN ACCESS

Edited by:

Stefano D'Errico,
Università degli Studi di Napoli
'Federico II', Italy

Reviewed by:

Ahmed Mahal,
University of Napoli, Italy
Nunzio Cardullo,
University of Catania, Italy

*Correspondence:

Dimitri Komiotis,
Laboratory of Bioorganic Chemistry,
Department of Biochemistry and
Biotechnology, University of Thessaly,
26 Ploutonos Str., 41221 Larissa,
Greece
dkom@bio.uth.gr

Specialty section:

This article was submitted to
Medicinal and Pharmaceutical
Chemistry, a section of the journal
Frontiers in Chemistry

Received: 04 December 2014

Accepted: 05 March 2015

Published: 23 March 2015

Citation:

Dimopoulou A, Manta S,
Parmenopoulou V, Kollatos N,
Christidou O, Triantakou VV,
Schols D and Komiotis D (2015) An
easy microwave-assisted synthesis of
C8-alkynyl adenine pyranonucleosides
as novel cytotoxic antitumor agents.
Front. Chem. 3:21.
doi: 10.3389/fchem.2015.00021

We describe the synthesis of C8-alkynyl adenine pyranonucleosides **4**, **5**, and 8-phenylethynyl-adenine (**II**), via Sonogashira cross-coupling reaction under microwave irradiation. Compounds **4e** and **II** were less cytostatic than 5-fluorouracil (almost an order of magnitude) against murine leukemia (L1210) and human cervix carcinoma (HeLa) cells, while the same compounds proved to be more active than 5-fluorouracil against human lymphocyte (CEM) cells.

Keywords: Sonogashira coupling reaction, 8-bromo-adenine, N⁶-benzoyladenine pyranonucleosides, microwave irradiation, cytotoxic activity

Introduction

Synthetic nucleoside analogs with modified nucleobase moieties are of considerable importance in the search for promising lead candidates endowed with antiviral, anticancer, and antibacterial activities (Herdewijn, 2008; Manta et al., 2014). Among them, a number of purine and pyrimidine substituted nucleoside derivatives exhibited activity in both solid tumors and hematological malignancies, behaving as antimetabolites, competing with physiological nucleosides, and consequently, interacting with a large number of intracellular targets to induce cytotoxicity (Hatse et al., 1999).

Alkynyl-modified nucleosides and especially pyrimidine derivatives substituted at C5 and purine derivatives substituted at C8, have been shown to possess interesting biological properties (Lin et al., 1985; Meneni et al., 2007; Lee et al., 2009; Vivet-Boudou et al., 2011). Some representative examples include 5-ethynyl-2'-deoxyuridine, which exhibited antiproliferative activity against human breast cancer cells, exceeding that of cisplatin and 5-fluorouracil, while 5-bromoethynyluridine demonstrated significant anti-HCV properties (Escuret et al., 2005; Meneni et al., 2007). Although, little effort has been made toward the synthesis of C8-modified purine nucleosides, in some cases, interesting biological properties have been reported, such as some 8-alkynyl adenosines, which proved to be very selective ligands for the A₃ adenosine receptor subtype behaving as adenosine antagonists (Volpini et al., 2001) and various C8-modified 2'-deoxy adenosines, which induced delayed chain termination *in vitro* and showed moderate anti HIV-1 activity in cell culture (Vivet-Boudou et al., 2011).

Considering all the progress made toward this direction, we have recently embarked on the synthesis of C5-substituted uracil and cytosine glucopyranonucleosides bearing a variety of alkyne substituents, such as linear alkyl chains and aromatic rings substituted with linear and branched alkyl groups (Dimopoulou et al., 2013), which effectively inhibited the proliferation of a variety of tumor cell lines and they also proved as some of the most potent inhibitors of the active site of glycogen phosphorylase (Kantsadi et al., 2012). Among these agents, the C5-phenylethynyluracil pyranonucleoside showed appreciable cytotoxic activity (IC_{50} of 5.2–6.2 μ M), comparable to 5-fluorouracil (Dimopoulou et al., 2013).

As a continuation of our studies on the synthesis of base-modified pyranonucleosides and considering the interesting biological properties of substituted purines, it was speculated that the introduction of alkynyl modifications at the 8-position of either adenine pyranonucleosides or even adenine itself, could possibly lead to more efficacious therapeutic agents. This conjugation appeared to us as a challenge and the first biological results confirmed our hypothesis.

Experimental (General Methods)

Melting points were recorded in a Mel-Temp apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on Merck precoated 60F254 plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or by charring with sulfuric acid. Flash column chromatography was performed using silica gel (240–400 mesh, Merck). 1H and ^{13}C NMR spectra were obtained at room temperature with a Bruker 300 spectrometer at 300 and 75.5 MHz, respectively using chloroform- d ($CDCl_3$) and dimethylsulfoxide- d_6 ($DMSO-d_6$) with internal tetramethylsilane (TMS). The 1H assignments were based on 1H - 1H COSY experiments executed using standard Varian software. Chemical shifts (δ) were given in ppm measured downfield from TMS, and spin-spin coupling constants are in Hz. Mass spectra were obtained on a ThermoQuest Finnigan AQA Mass Spectrometer (electrospray ionization). Optical rotations were measured using an Autopol I polarimeter and UV-Vis spectra were recorded on a PG T70 UV-VIS spectrometer. Acetonitrile (CH_3CN) was distilled from calcium hydride and stored over 3 Å molecular sieves. N,N -Dimethylformamide (DMF) was stored over 3 Å molecular sieves. All reactions sensitive to oxygen or moisture were carried out under nitrogen atmosphere using oven-dried glassware. All microwave irradiation experiments were carried out in a dedicated CEM-Explorer and CEM Discover monomode microwave apparatus, operating at a frequency of 2.45 GHz with continuous irradiation power from 0 to 300 W with utilization of the standard absorbance level of 300 W maximum power. The reactions were carried out in 10-mL glass tubes, sealed with a Teflon septum and placed in the microwave cavity. Initially, microwave irradiation of required watts was used, and the temperature was ramped from room temperature to the desired temperature. Once this was reached the reaction mixture was held at this temperature for the required time. The reaction mixture was continuously stirred during the reaction. The temperature was measured with an IR sensor on

the outer surface of the process vial. After the irradiation period, gas jet cooling rapidly cooled the reaction vessel to ambient temperature.

9-(2',3',4',6'-Tetra-O-Acetyl- β -D-Glucopyranosyl)- N^6 -Benzoyl Adenine (2)

A mixture of N^6 -benzoyladenine (797 mg, 3.33 mmol, 1.3 equiv), hexamethyldisilazane (HMDS) (871 μ L, 4.13 mmol, 1.24 equiv) and saccharine (27 mg, 0.15 mmol, 0.046 equiv) in anhydrous CH_3CN (14 mL) was refluxed for 1 h under nitrogen. 1,2,3,4,6-Penta-O-acetyl-D-glucopyranose (**1**) (1 g, 2.56 mmol) and tin(IV) chloride ($SnCl_4$) (419 μ L, 3.58 mmol, 1.4 equiv) were then added and the reaction mixture was stirring under reflux for 2 more h, cooled, neutralized with aqueous sodium bicarbonate, and extracted with ethyl acetate (1000 mL). The organic extract was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue was purified by flash column chromatography (EtOAc/ hexane 7:3) to give compound **2**, as a white solid (814 mg, 56%); mp 169–171°C; $[\alpha]_D^{25} = -6$ (c 0.2, $CHCl_3$); $R_f = 0.47$ (EtOAc); λ_{max} 280 nm (ϵ 20557); 1H NMR ($CDCl_3$, 300 MHz): δ 8.83 (s, 1H, H-2), 8.22 (s, 1H, H-8), 8.04–7.51 (m, 5H, Bz), 5.98 (d, 1H, $J_{1',2'} = 9.4$ Hz, H-1'), 5.65 (t, 1H, $J = 9.3$ Hz, H-3'), 5.50 (t, 1H, $J = 9.4$ Hz, H-2'), 5.32 (t, 1H, $J = 9.7$ Hz, H-4'), 4.34 (dd, 1H, $J_{5',6a'} = 4.1$ Hz, $J_{6a',6b'} = 12.8$ Hz, H-6a'), 4.24–4.14 (m, 1H, H-6b'), 4.11–4.02 (m, 1H, H-5'), 2.08, 2.07, 2.04, 1.79 (4s, 12H, 4OAc); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.4, 169.8, 169.3, 169.0, 164.5, 152.0, 151.9, 149.5, 140.9, 133.1, 133.0, 128.9, 128.1, 122.2, 80.6, 75.2, 72.7, 70.3, 67.7, 61.5, 20.6, 20.5, 20.4, 20.1; Mass ($M+H$) $^+$: 570.16; Anal. Calcd. for $C_{26}H_{27}N_5O_{10}$: C, 54.83; H, 4.78; N, 12.30%. Found: C, 55.16; H, 4.66; N, 12.53%.

9-(2',3',4',6'-Tetra-O-Acetyl- β -D-Glucopyranosyl)-8-Bromo- N^6 -Benzoyl Adenine (3)

To a solution of 9-(2',3',4',6'-tetra-O-acetyl- β -D-glucopyranosyl)- N^6 -benzoyl adenine (**2**) (250 mg, 0.44 mmol), and sodium acetate (172 mg, 2.1 mmol) in 1.6 mL of glacial acetic acid, 75 μ L, 1.45 mmol of bromine were added. The reaction was left to stir at room temperature until completion (14 h). The whole was extracted with ethyl acetate and saturated $Na_2S_2O_3$. The organic layer was washed with water and brine, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue was purified by flash column chromatography (EtOAc/ hexane 7:3) to give compound **3**, (171 mg, 60%); $[\alpha]_D^{25} = -3$ (c 0.2, $CHCl_3$); $R_f = 0.45$ (EtOAc/ hexane 7:3); λ_{max} 282 nm (ϵ 21223); 1H NMR ($CDCl_3$, 300 MHz): δ 8.82 (s, 1H, H-2), 8.04–7.49 (m, 5H, Bz), 6.21 (t, 1H, $J = 9.6$ Hz, H-2'), 5.96 (d, 1H, $J_{1',2'} = 9.6$ Hz, H-1'), 5.47 (t, 1H, $J = 9.4$ Hz, H-3'), 5.35 (t, 1H, $J = 9.9$ Hz, H-4'), 4.26–4.25 (m, 2H, H-6a', H-6b'), 4.06–3.96 (m, 1H, H-5'), 2.09, 2.07, 2.04, 1.79 (4s, 12H, 4OAc); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.5, 170.0, 169.9, 169.3, 164.7, 153.3, 152.9, 149.2, 133.3, 131.0, 128.9, 128.6, 128.2, 122.3, 78.2, 72.9, 68.8, 67.6, 67.5, 61.5, 20.7, 20.6, 20.5, 20.2; Mass ($M+H$) $^+$: 648.08; Anal. Calcd. for $C_{26}H_{26}BrN_5O_{10}$: C, 48.16; H, 4.04; Br, 12.32; N, 10.80%. Found: C, 48.44; H, 4.15; Br, 12.11; N, 10.99%.

General Experimental Procedure for the Preparation of Protected C8-Alkynyl Adenine Pyranonucleosides (4a–e)

The appropriate alkynes (3 equiv), Pd(PPh₃)₄ (43 mg, 0.1 equiv), CuI (7 mg, 0.1 equiv), triethylamine (103 μ L, 2 equiv) and 9-(2',3',4',6'-tetra-O-acetyl- β -D-glucopyranosyl)-8-bromo-N⁶-benzoyl adenine (3) (240 mg, 0.37 mmol), were irradiated under microwaves (200 W) in 1 mL of anhydrous DMF for 10 min at 120°C. The reaction mixture was concentrated under reduced pressure and the crude residue was purified by flash chromatography on silica gel. The purified material was dried *in vacuo* to afford the corresponding derivatives 4a–e, as colorless foams.

9-(2',3',4',6'-Tetra-O-Acetyl- β -D-Glucopyranosyl)-8-Heptynyl-N⁶-Benzoyl Adenine (4a)

139 mg, 56%; $[\alpha]_D^{22} = -4$ (c 0.4, CHCl₃); $R_f = 0.10$ (EtOAc/hexane 6:4); λ_{\max} 303 nm (ϵ 22594); ¹H NMR (CDCl₃, 300 MHz): δ 8.99 (br s, 1H, NH), 8.85 (s, 1H, H-2), 8.03–7.41 (m, 5H, Bz), 6.28 (t, 1H, $J = 9.4$ Hz, H-2'), 5.95 (d, 1H, $J_{1',2'} = 9.2$ Hz, H-1'), 5.46 (t, 1H, $J = 9.3$ Hz, H-3'), 5.35 (t, 1H, $J = 9.3$ Hz, H-4'), 4.28–4.19 (m, 2H, H-6a', H-6b'), 4.04–3.87 (m, 1H, H-5'), 2.62 (t, 2H, $J = 7.1$ Hz, α -CH₂), 2.09, 2.08, 2.05, 1.78 (4s, 12H, 4OAc), 1.56–1.35 (m, 6H, 3 \times CH₂), 0.96 (t, 3H, $J = 7.2$ Hz, CH₃); ¹³C NMR (CDCl₃, 75.5 MHz): δ 170.8, 170.5, 170.0, 169.8, 165.0, 153.5, 152.2, 151.3, 148.8, 134.9, 133.0, 129.2, 127.9, 119.9, 97.7, 94.5, 82.9, 70.8, 70.1, 67.5, 67.2, 63.7, 31.5, 31.2, 29.6, 22.3, 21.5, 21.3, 21.0, 20.9, 15.2; Mass (M+H)⁺: 664.26; Anal. Calcd. for C₃₃H₃₇N₅O₁₀: C, 59.72; H, 5.62; N, 10.55%. Found: C, 59.96; H, 5.94; N, 10.65%.

9-(2',3',4',6'-Tetra-O-Acetyl- β -D-Glucopyranosyl)-8-Phenylethynyl-N⁶-Benzoyl Adenine (4b)

161 mg, 66%; $[\alpha]_D^{22} = -6$ (c 0.4, CHCl₃); $R_f = 0.33$ (CH₂Cl₂/MeOH 9.8:0.2); λ_{\max} 300 nm (ϵ 29706); ¹H NMR (CDCl₃, 300 MHz): δ 8.95 (br s, 1H, NH), 8.87 (s, 1H, H-2), 8.01–7.45 (m, 10H, Bz and Ph), 6.31 (t, 1H, $J = 9.3$ Hz, H-2'), 6.10 (d, 1H, $J_{1',2'} = 9.4$ Hz, H-1'), 5.49 (t, 1H, $J = 9.2$ Hz, H-3'), 5.35 (t, 1H, $J = 9.5$ Hz, H-4'), 4.31–4.22 (m, 2H, H-6a', H-6b'), 4.08–3.98 (m, 1H, H-5'), 2.09, 2.05, 1.90, 1.77 (4s, 12H, 4OAc); ¹³C NMR (CDCl₃, 75.5 MHz): δ 170.6, 170.3, 169.9, 169.7, 165.3, 153.1, 152.4, 150.5, 148.5, 134.5, 133.8, 133.5, 129.9, 129.5, 129.0, 128.3, 123.6, 119.3, 102.6, 98.7, 87.3, 83.6, 70.5, 67.9, 67.5, 63.5, 21.5, 21.3, 20.9, 20.5; Mass (M+H)⁺: 670.18; Anal. Calcd. for C₃₄H₃₁N₅O₁₀: C, 60.98; H, 4.67; N, 10.46%. Found: C, 61.23; H, 4.28; N, 10.80%.

9-(2',3',4',6'-Tetra-O-Acetyl- β -D-Glucopyranosyl)-8-p-Tolylethynyl-N⁶-Benzoyl Adenine (4c)

157 mg, 61%; $[\alpha]_D^{22} = -10$ (c 0.3, CHCl₃); $R_f = 0.37$ (EtOAc/hexane 8:2); λ_{\max} 325 nm (ϵ 27336); ¹H NMR (CDCl₃, 300 MHz): δ 8.98 (br s, 1H, NH), 8.86 (s, 1H, H-2), 8.02–7.29 (m, 9H, Bz and ArH), 6.31 (t, 1H, $J = 9.4$ Hz, H-2'), 6.08 (d, 1H, $J_{1',2'} = 9.3$ Hz, H-1'), 5.48 (t, 1H, $J = 9.4$ Hz, H-3'), 5.37 (t, 1H, $J = 9.7$ Hz,

H-4'), 4.29–4.22 (m, 2H, H-6a', H-6b'), 4.07–3.98 (m, 1H, H-5'), 2.43 (s, 3H, CH₃), 2.09, 2.05, 1.91, 1.77 (4s, 12H, 4OAc); ¹³C NMR (CDCl₃, 75.5 MHz): δ 170.3, 170.1, 169.7, 169.3, 165.1, 153.2, 152.3, 150.7, 148.7, 139.5, 135.8, 133.7, 133.4, 129.6, 129.2, 128.8, 120.3, 120.1, 102.4, 98.3, 87.6, 83.5, 70.7, 67.5, 67.1, 63.7, 22.5, 21.7, 21.5, 21.0, 20.2; Mass (M+H)⁺: 684.22; Anal. Calcd. for C₃₅H₃₃N₅O₁₀: C, 61.49; H, 4.87; N, 10.24%. Found: C, 61.84; H, 4.52; N, 10.02%.

9-(2',3',4',6'-Tetra-O-Acetyl- β -D-Glucopyranosyl)-8-(Pyridin-3-yl-Ethynyl)-N⁶-Benzoyl Adenine (4d)

141 mg, 58%; $[\alpha]_D^{22} = -12$ (c 0.2, CHCl₃); $R_f = 0.23$ (EtOAc/hexane 6:4); λ_{\max} 320 nm (ϵ 16156); ¹H NMR (CDCl₃, 300 MHz): δ 8.99 (br s, 1H, NH), 8.87 (s, 1H, H-2), 8.72–7.39 (m, 9H, Bz and pyridine), 6.26 (t, 1H, $J = 9.5$ Hz, H-2'), 6.11 (d, 1H, $J_{1',2'} = 9.3$ Hz, H-1'), 5.48 (t, 1H, $J = 9.3$ Hz, H-3'), 5.35 (t, 1H, $J = 9.7$ Hz, H-4'), 4.32 (dd, 1H, $J_{5',6a'} = 4.7$ Hz, $J_{6a',6b'} = 12.5$ Hz, H-6a'), 4.21 (dd, 1H, $J_{5',6b'} = 2.0$ Hz, $J_{6a',6b'} = 12.5$ Hz, H-6b'), 4.09–4.00 (m, 1H, H-5'), 2.09, 2.05, 1.94, 1.78 (4s, 12H, 4OAc); ¹³C NMR (CDCl₃, 75.5 MHz): δ 170.4, 170.0, 169.6, 169.2, 165.4, 153.0, 152.7, 151.6, 150.7, 150.5, 148.8, 140.5, 135.4, 133.6, 129.5, 128.2, 124.6, 120.5, 117.1, 98.7, 95.6, 83.3, 75.7, 70.5, 67.5, 67.2, 63.5, 21.3, 21.0, 20.9, 20.7; Mass (M+H)⁺: 671.21; Anal. Calcd. for C₃₃H₃₀N₆O₁₀: C, 59.10; H, 4.51; N, 12.53%. Found: C, 59.42; H, 4.76; N, 12.22%.

9-(2',3',4',6'-Tetra-O-Acetyl- β -D-Glucopyranosyl)-8-(Pyridin-2-yl-Ethynyl)-N⁶-Benzoyl Adenine (4e)

127 mg, 50%; $[\alpha]_D^{22} = -2$ (c 0.1, CHCl₃); $R_f = 0.21$ (EtOAc/hexane 7:3); λ_{\max} 315 nm (ϵ 22103); ¹H NMR (CDCl₃, 300 MHz): δ 8.80 (br s, 2H, NH, H-2), 8.07–7.35 (m, 9H, Bz and pyridine), 6.31 (t, 1H, $J = 9.4$ Hz, H-2'), 6.12 (d, 1H, $J_{1',2'} = 9.3$ Hz, H-1'), 5.71 (t, 1H, $J = 9.5$ Hz, H-3'), 5.48 (t, 1H, $J = 9.5$ Hz, H-4'), 4.29–4.25 (m, 2H, H-6a', H-6b'), 4.10–4.02 (m, 1H, H-5'), 2.09, 2.03, 1.92, 1.75 (4s, 12H, 4OAc); ¹³C NMR (CDCl₃, 75.5 MHz): δ 170.6, 170.3, 169.7, 169.5, 164.3, 153.2, 152.8, 151.5, 149.9, 148.3, 145.2, 139.5, 134.6, 132.7, 128.6, 127.8, 127.6, 123.7, 119.7, 95.7, 92.5, 84.8, 82.7, 72.8, 69.5, 68.3, 65.7, 21.0, 20.7, 20.6, 20.5; Mass (M+H)⁺: 671.20; Anal. Calcd. for C₃₃H₃₀N₆O₁₀: C, 59.10; H, 4.51; N, 12.53%. Found: C, 59.40; H, 4.73; N, 12.34%.

General Procedure for the Preparation of Unprotected C8-Alkynyl Adenine Pyranonucleosides (5a–d)

The protected nucleosides 4a–e (0.5 mmol), were treated with methanolic ammonia (saturated at 0°C, 27.9 mL). The solution was stirred overnight at room temperature and then evaporated under reduced pressure. The residue was purified by flash column chromatography to afford the unprotected derivatives 5a–d, in 60–72% yields, as yellowish and white foams.

9-(β -D-Glucopyranosyl)-8-Heptynyl-Adenine (5a)

117 mg, 60%; $[\alpha]_D^{22} = -2$ (c 0.2, MeOH); $R_f = 0.17$ (EtOAc/MeOH 8:2) λ_{\max} 292 nm (ϵ 19971); ¹H NMR (DMSO-*d*₆,

300 MHz): δ 8.15 (s, 1H, H-2), 7.24 (s, 2H, NH₂), 5.43 (d, 1H, $J_{1',2'} = 9.5$ Hz, H-1'), 5.16, 5.09, 5.00 (3br s, 3H, 3OH), 4.60-4.45 (m, 2H, OH, H-2'), 3.81-3.34 (m, 5H, H-3', H-4', H-5', H-6a', H-6b'), 1.78-1.76 (m, 2H, β -CH₂), 1.63 (t, 2H, $J = 7.2$ Hz, α -CH₂), 1.51-1.30 (m, 4H, 2 \times CH₂), 0.93 (t, 3H, $J = 7.2$ Hz, CH₃). ¹³C NMR (DMSO-*d*₆, 75.5 MHz): δ 156.8, 152.7, 150.1, 148.3, 119.9, 96.2, 93.9, 83.2, 78.8, 73.6, 70.5, 66.7, 65.4, 30.9, 29.2, 22.3, 19.0, 14.5; Mass (M+H)⁺: 392.20; Anal. Calcd. for C₁₈H₂₅N₅O₅: C, 55.23; H, 6.44; N, 17.89%. Found: C, 54.90; H, 6.73; N, 17.72%.

9-(β -D-Glucopyranosyl)-8-Phenylethynyl-Adenine (5b)

143 mg, 72%; $[\alpha]_D^{22} = -6$ (c 0.1, MeOH); $R_f = 0.30$ (EtOAc/MeOH 9:1); λ_{\max} 313 nm (ϵ 14209); ¹H NMR (DMSO-*d*₆, 300 MHz): δ 8.19 (s, 1H, H-2), 7.66 (br s, 2H, NH₂), 7.61-7.47 (m, 5H, Ph), 5.55 (d, 1H, $J_{1',2'} = 9.2$ Hz, H-1'), 5.40 (d, 1H, $J = 5.3$ Hz, OH), 5.34 (d, 1H, $J = 4.1$ Hz, OH), 5.21 (d, 1H, $J = 5.1$ Hz, OH), 4.70 (t, 1H, $J = 4.4$ Hz, OH), 4.54-4.40 (m, 1H, H-2'), 3.81-3.39 (m, 5H, H-3', H-4', H-5', H-6a', H-6b'). ¹³C NMR (DMSO-*d*₆, 75.5 MHz): δ 156.3, 152.6, 150.0, 148.1, 132.7, 128.9, 128.7, 123.0, 119.7, 101.9, 96.3, 86.8, 83.3, 78.1, 73.4, 70.8, 65.8; Mass (M+H)⁺: 398.15; Anal. Calcd. for C₁₉H₁₉N₅O₅: C, 57.43; H, 4.82; N, 17.62%. Found: C, 57.81; H, 4.51; N, 17.90%.

9-(β -D-Glucopyranosyl)-8-*p*-Tolylethynyl-Adenine (5c)

140 mg, 67%; $[\alpha]_D^{22} = -4$ (c 0.1, MeOH); $R_f = 0.21$ (EtOAc/MeOH 8:2); λ_{\max} 316 nm (ϵ 12871); ¹H NMR (DMSO-*d*₆, 300 MHz): δ 8.18 (s, 1H, H-2), 7.55 (d, 2H, $J = 8.1$ Hz, ArH), 7.37 (br s, 2H, NH₂), 7.32 (d, 2H, $J = 8.0$ Hz, ArH), 5.55 (d, 1H, $J_{1',2'} = 9.3$ Hz, H-1'), 5.25 (d, 1H, $J = 4.1$ Hz, OH), 5.15, 5.04 (2br s, 2H, 2OH), 4.58-4.47 (m, 2H, OH, H-2'), 3.81-3.34 (m, 5H, H-3', H-4', H-5', H-6a', H-6b'), 2.38 (s, 3H, CH₃). ¹³C NMR (DMSO-*d*₆, 75.5 MHz): δ 156.4, 152.1, 150.4, 148.5, 138.5, 132.5, 129.0, 122.3, 120.2, 101.8, 96.0, 86.9, 83.0, 78.9, 73.5, 70.7, 65.6, 24.6; Mass (M+H)⁺: 412.17; Anal. Calcd. for C₂₀H₂₁N₅O₅: C, 58.39; H, 5.14; N, 17.02%. Found: C, 58.72; H, 5.45; N, 17.27%.

9-(β -D-Glucopyranosyl)-8-(Pyridin-3-yl-Ethynyl)-Adenine (5d)

139 mg, 70%; $[\alpha]_D^{22} = +2$ (c 0.2, MeOH); $R_f = 0.16$ (CH₂Cl₂/MeOH 7:3); λ_{\max} 306 nm (ϵ 7353); ¹H NMR (DMSO-*d*₆, 300 MHz): δ 8.89-8.68 (m, 2H, pyridine), 8.21 (s, 1H, H-2), 8.10-7.52 (m, 2H, pyridine), 7.44 (br s, 2H, NH₂), 5.58 (d, 1H, $J_{1',2'} = 9.3$ Hz, H-1'), 5.28, 5.07, 4.56 (3 br s, 3H, 3OH), 4.45 (t, 1H, $J = 8.1$ Hz, OH), 3.81-3.40 (m, 6H, H-2', H-3', H-4', H-5', H-6a', H-6b'). ¹³C NMR (DMSO-*d*₆, 75.5 MHz): δ 156.9, 152.5, 152.3, 150.5, 149.8, 148.5, 139.9, 123.8, 119.8, 116.8, 96.0, 95.7, 83.1, 78.7, 74.1, 73.9, 70.2, 65.2; Mass (M+H)⁺: 399.15; Anal. Calcd. for C₁₈H₁₈N₆O₅: C, 54.27; H, 4.55; N, 21.10%. Found: C, 54.62; H, 4.74; N, 21.38%.

8-Phenylethynyl-Adenine (II)

8-Bromoadenine (I) (43 mg, 0.2 mmol) was mixed with anhydrous DMF (1 mL), phenylacetylene (66 μ L, 3 equiv),

triethylamine (55 μ L, 2 equiv), Pd(PPh₃)₄ (23 mg, 0.1 equiv), CuI (11 mg, 0.3 equiv) and irradiated with microwaves (200 W) for 6 min at 60°C. After removing volatiles *in vacuo*, the solid residue was purified by flash chromatography (CH₂Cl₂/MeOH, 9.5:0.5) to provide compound II, as yellowish foam. 19 mg, 40%; $[\alpha]_D^{22} = -2$ (c 0.2, MeOH); $R_f = 0.13$ (CH₂Cl₂/MeOH 8.5:1.5); λ_{\max} 308 nm (ϵ 9701); ¹H NMR (DMSO-*d*₆, 300 MHz): δ 13.38 (br s, 1H, NH), 8.15 (s, 1H, H-2), 7.65-7.49 (m, 5H, Ph), 7.27 (br s, 2H, NH₂). ¹³C NMR (DMSO-*d*₆, 75.5 MHz): δ 156.8, 154.2, 152.6, 148.1, 132.7, 129.1, 129.0, 123.0, 119.8, 101.8, 67.1; Mass (M+H)⁺: 236.08; Anal. Calcd. for C₁₃H₉N₅: C, 66.37; H, 3.86; N, 29.77%. Found: C, 66.59; H, 4.01; N, 29.40%.

Antiproliferative Assay

Compounds 4e, 5a-d, and II, were evaluated for their cytostatic activity against human cervix carcinoma (HeLa) cells, human lymphocytes (CEM) as well as murine leukemia (L1210) cells. All assays were performed in 96-well microtiter plates. To each well (5–7.5) $\times 10^4$ tumor cells were added and varying concentrations of the test compounds ranging from 250, 50, 10, 2, 0.4, to 0.08 μ M. The tumor cells were allowed then to proliferate at 37°C in a humidified CO₂-controlled atmosphere. To obtain their optimal growth curves this is for 2 days of the murine leukemia (L1210) cells and for 3 days for the human lymphocytic (CEM) cells and the human cervix carcinoma (HeLa) cells. At the end of the incubation period, the cells were counted in a Coulter counter. The IC₅₀ (50% inhibitory concentration) was defined as the concentration of the compound that inhibited cell proliferation by 50%. Experiments were repeated at least three times and these data are presented in Table 1.

Results and Discussion

Herein, we describe the synthesis of C8-alkynyl adenine pyranonucleosides and present their biological properties. The starting material of our synthesis was the commercially available per-*O*-acetylated D-glucose 1 which upon coupling, *via* two-step Vorbrüggen method (Vorbrüggen and Höfle, 1981),

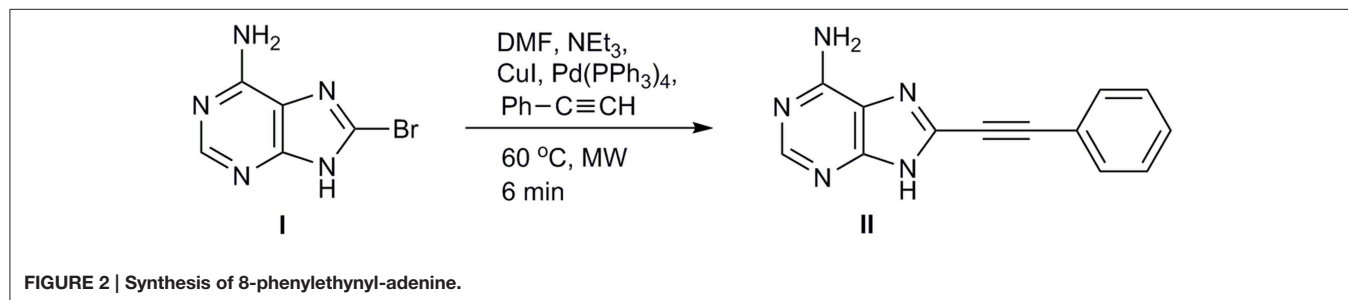
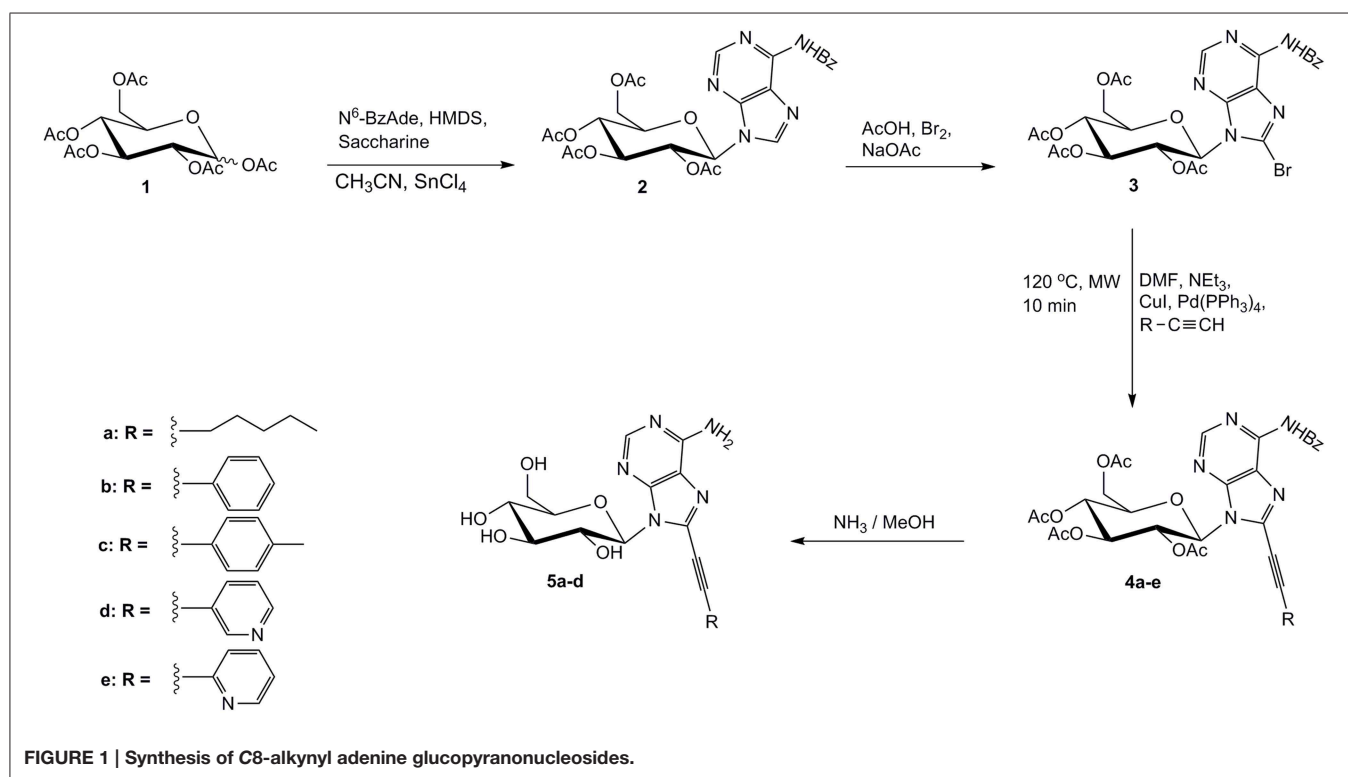
TABLE 1 | Cytostatic activity of the compounds against tumor cell (L1210, CEM, and HeLa) proliferation.

| Compound | IC ₅₀ ^a (μ M) | | |
|----------------|--|---------------|-----------------|
| | L1210 | CEM | HeLa |
| 4e | 2.9 \pm 0.0 | 1.2 \pm 0.2 | 3.0 \pm 0.7 |
| 5a | >250 | >250 | >250 |
| 5b | >250 | >250 | >250 |
| 5c | >250 | >250 | >250 |
| 5d | >250 | >250 | \geq 250 |
| II | 5.9 \pm 5.5 | 4.2 \pm 0.8 | 10 \pm 3.0 |
| 5-Fluorouracil | 0.33 \pm 0.17 | 18 \pm 5 | 0.54 \pm 0.12 |

^a50% inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%.

with *N*⁶-benzoyl adenine, gave selectively the *N*9-isomeric adenine nucleoside **2**, under thermodynamically controlled conditions ($\text{SnCl}_4/\text{CH}_3\text{CN}$, reflux). Trans rule was followed (Baker, 1957) and the β -configured nucleoside **2** was solely obtained as deduced from ^1H NMR vicinal coupling data ($J_{1',2'} = 9.4 \text{ Hz}$). Since halogenated C8-purine nucleosides have proven useful intermediates for the efficient preparation of their corresponding C8-alkynyl derivatives (Agrofoglio et al., 2003), the adenine nucleoside **2** was directly brominated after treatment with $\text{Br}_2/\text{AcOH}/\text{AcONa}$ (Holmes and Robins, 1964), affording the 8-bromo analog **3**, in approximately 60% yield after aqueous work-up and flash chromatography. The position of substitution of the bromine atom was further confirmed by the absence of the characteristic sharp absorption peak at 8.22 ppm due to H-8 of nucleoside **2**, while the syn conformation for adenine was induced due to the bulky bromo substituent at C8 position (Sarma et al., 1974). 8-Alkynyl

adenine nucleosides **4** were accessed, through Sonogashira cross-coupling reaction of intermediate **3** with several terminal acetylenes, under microwave irradiation (200 W) (**Figure 1**). In a typical experiment, an effective catalyst ($\text{Pd}(\text{PPh}_3)_4$)/co-catalyst (CuI) combination proved to be (1:1) ratio (Meneni et al., 2007) affording **4a–e**, in satisfactory yields (50–66%). To provide a detailed structure-activity relationship studies, diverse alkyne substituents *R* were selected which include linear alkyl chains (**4a**, *R* = *n*-pentyl), aromatic rings (**4b**, *R* = phenyl, **4c**, *R* = *p*-tolyl) and pyridine moieties (**4d**, *R* = 3-pyridyl, **4e**, *R* = 2-pyridyl). Finally, total deprotection of **4a–e** by the action of saturated methanolic ammonia afforded only the target derivatives **5a–d**, while attempts to remove all protecting groups from **4e** either with sodium methoxide (Bozó et al., 1998) or potassium carbonate-methanol (Plattner et al., 1972) resulted in a mixture of intractable and inseparable materials.



Based on the promising cytotoxic activity profile of our prior synthesized C5-phenylethynyl uracil glucopyranonucleoside (Dimopoulou et al., 2013) and in order to explore the impact of the glycosidic part on the biological activity as well as the potential inhibitory effects of adenine moiety, we sought to introduce phenylacetylene substituent in 8-position of adenine itself. Therefore, we investigated the development of the efficient Sonogashira alkylation protocol for the cross-coupling of commercially available 8-bromoadenine (**I**) with phenylacetylene under microwave irradiation (200 W) (Figure 2). 8-Bromoadenine (**I**) was mixed with anhydrous DMF, phenylacetylene, triethylamine, Pd(PPh₃)₄, CuI, irradiated with microwaves for 6 min at 60°C and after removing volatiles *in vacuo*, the solid residue was purified by flash chromatography to provide compound **II**, in acceptable yield (40%).

Cytostatic Activity

The cytostatic activity of the novel C8-modified adenine pyranonucleosides **4e**, **5a–d** as well as 8-phenylethynyl-adenine (**II**) was determined against murine leukemia (L1210), human lymphocyte (CEM) and human cervix carcinoma (HeLa) cell cultures (Table 1). Compounds **4e** and **II** were less cytostatic than 5-fluorouracil (almost an order of magnitude) against murine leukemia (L1210) and human cervix carcinoma (HeLa) cells, while the same compounds proved to be more active than 5-fluorouracil against human lymphocyte (CEM) cells.

References

- Agrofoglio, L. A., Gillaizeau, I., and Saito, Y. (2003). Palladium-assisted routes to nucleosides. *Chem. Rev.* 103, 1875–1916. doi: 10.1021/cr010374q
- Baker, B. R. (1957). "Stereochemistry of nucleoside synthesis," in *Ciba Foundation Symposium on the Chemistry and Biology of Purines*, eds G. E. W. Wohlstenholme and C. M. O'Connor (London: John Wiley & Sons), 120–129.
- Bozó, E., Boros, S., Kuszmann, J., Gács-Baitz, E., and Párkányi, L. (1998). An economic synthesis of 1,2,3,4-tetra-O-acetyl-5-thio-D-xylopyranose and its transformation into 4-substituted-phenyl 1,5-dithio-D-xylopyranosides possessing antithrombotic activity. *Carbohydr. Res.* 308, 297–310. doi: 10.1016/S0008-6215(98)00088-3
- Dimopoulou, A., Manta, S., Kiritsis, C., Gkaragkouni, D. N., Papasotiriou, I., Balzarini, J., et al. (2013). Rapid microwave-enhanced synthesis of C5-alkynyl pyranonucleosides as novel cytotoxic antitumor agents. *Bioorg. Med. Chem. Lett.* 23, 1330–1333. doi: 10.1016/j.bmcl.2012.12.092
- Escuret, V., Aucagne, V., Joubert, N., Durantel, D., Rapp, L. K., Schinazi, F. R., et al. (2005). Synthesis of 5-haloethynyl- and 5-(1,2-dihalo)vinyluracil nucleosides: antiviral activity and cellular toxicity. *Bioorg. Med. Chem.* 13, 6015–6024. doi: 10.1016/j.bmc.2005.06.021
- Hatse, S., De Clercq, E., and Balzarini, J. (1999). Role of antimetabolites of purine and pyrimidine nucleotide metabolism in tumor cell differentiation. *Biochem. Pharmacol.* 58, 539–555. doi: 10.1016/S0006-2952(99)00035-0
- Herdevijn, P. (2008). *Modified Nucleosides in Biochemistry, Biotechnology and Medicine*. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA.
- Holmes, E. R., and Robins, K. R. (1964). Purine nucleosides. VII. Direct bromination of adenosine, deoxyadenosine, guanosine, and related purine nucleosides. *J. Am. Chem. Soc.* 86, 1242–1245. doi: 10.1021/ja01060a057
- Kantsadi, A. L., Manta, S., Psara, A. M., Dimopoulou, A., Kiritsis, C., Parmenopoulou, V., et al. (2012). The binding of C5-alkynyl and alkylfurano[2,3-d]pyrimidine glucopyranonucleosides to glycogen phosphorylase b: synthesis, biochemical and biological assessment. *Eur. J. Med. Chem.* 54, 740–749. doi: 10.1016/j.ejmech.2012.06.029
- Lee, Y. S., Park, S. M., Kim, H. M., Park, S. K., Lee, K., Lee, C. W., et al. (2009). C5-Modified nucleosides exhibiting anticancer activity. *Bioorg. Med. Chem. Lett.* 19, 4688–4691. doi: 10.1016/j.bmcl.2009.06.072
- Lin, T. S., Cheng, J. C., Ishiguro, K., and Sartorelli, A. C. (1985). Purine and 8-substituted purine arabinofuranosyl and ribofuranosyl nucleoside derivatives as potential inducers of the differentiation of the Friend erythroleukemia. *J. Med. Chem.* 28, 1481–1485. doi: 10.1021/jm00148a018
- Manta, S., Kiritsis, C., Dimopoulou, A., Parmenopoulou, V., Kollatos, N., Tsotinis, A., et al. (2014). Unsaturation: an important structural feature to nucleosides' antiviral activity. *Anti Infect. Agents* 12, 2–57. doi: 10.2174/22113525113119990106
- Meneni, S., Ott, I., Sergeant, C. D., Sniady, A., Gust, R., and Dembinski, R. (2007). 5-Alkynyl-2'-deoxyuridines: chromatography-free synthesis and cytotoxicity evaluation against human breast cancer cells. *Bioorg. Med. Chem.* 15, 3082–3088. doi: 10.1016/j.bmc.2007.01.048
- Plattner, J. J., Gless, R. D., and Rapoport, H. (1972). Synthesis of some DE and CDE ring analogs of camptothecin. *J. Am. Chem. Soc.* 94, 8613–8615. doi: 10.1021/ja00779a072
- Sarma, H. R., Lee, C.-H., Evans, E. F., Yathindra, N., and Sundaralingam, M. (1974). Probing the interrelation between the glycosyl torsion, sugar pucker, and the backbone conformation in C(8) substituted adenine nucleotides by ¹H and ¹H-³¹P fast fourier transform nuclear magnetic resonance methods and conformational energy calculations. *J. Am. Chem. Soc.* 96, 7337–7348. doi: 10.1021/ja00830a028

Conclusion

In summary, we have prepared several novel C8-alkynyl adenine nucleosides as well as 8-phenylethynyl-adenine, *via* Sonogashira coupling conditions under microwave irradiation. Among the compound series tested, the protected adenine pyranonucleoside **4e**, as well as phenylethynyl adenine (**II**) showed significant cytotoxicity (IC₅₀ of 1.2–10.0 μM) against murine leukemia (L1210), human lymphocyte (CEM) and human cervix carcinoma (HeLa) cell cultures. Since the glucose derivative of phenylethynyl adenine, nucleoside **5b**, showed no activity, it is clear that it is stable and not susceptible to hydrolysis. The replacement of glucose with ribo, arabino, and deoxyribose moieties as well as the introduction of functional substituents on the phenyl ring, such as halogens, nitro or amino and alkyl groups, could be explored in the future in an attempt to further increase the cytostatic potential of these lead compounds.

Acknowledgments

This work was supported in part by the Postgraduate Programmes "Biotechnology-Quality assessment in Nutrition and the Environment", "Application of Molecular Biology-Molecular Genetics- Molecular Markers", Department of Biochemistry and Biotechnology, University of Thessaly, and the KU Leuven (GOA 10/14). We thank Lizette van Berckelaer for her excellent technical assistance in the biological assays.

- Vivet-Boudou, V., Isel, C., Sleiman, M., Smyth, R., Ben Gaied, N., Barhoum, P., et al. (2011). 8-Modified-2'-deoxyadenosine analogues induce delayed polymerization arrest during HIV-1 reverse transcription. *PLoS ONE* 6:e27456. doi: 10.1371/journal.pone.0027456
- Volpini, R., Costanzi, S., Lambertucci, C., Vittori, S., Klotz, K., Lorenzen, A., et al. (2001). Introduction of alkynyl chains on C-8 of adenosine led to very selective antagonists of the A(3) adenosine receptor. *Bioorg. Med. Chem. Lett.* 11, 1931–1934. doi: 10.1016/S0960-894X(01)00347-X
- Vorbrüggen, H., and Höfle, G. (1981). Nucleosides syntheses XXII: on the mechanism of the nucleoside synthesis. *Chem. Ber.* 114, 1256–1268. doi: 10.1002/cber.19811140405

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2015 Dimopoulou, Manta, Parmenopoulou, Kollatos, Christidou, Triantakoustanti, Schols and Komiotis. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.